p=0.046 IL4 vs. IL-1 in non compressed samples, not significant after compression) (Figure). Supernatant GAG content, marker of matrix remodeling, appeared unaffected by the treatment.

Conclusions: Mechanical stimulation plays a central role in the maintenance of cartilage homeostasis, but is also involved in the pathogenesis of OA. Our data seem to indicate that physiological compression of OA human cartilage tissue could counteract the effect of the inflammatory milieu by modulating cartilage matrix component metabolism. These data stimulate further studies to better elucidate the role of mechantransduction on cartilage behaviour both in normal and pathologic conditions. It is of particular interest the use of healthy human cartilage, where the absence of an altered tissue homeostasis could better highlight the effect of loading.

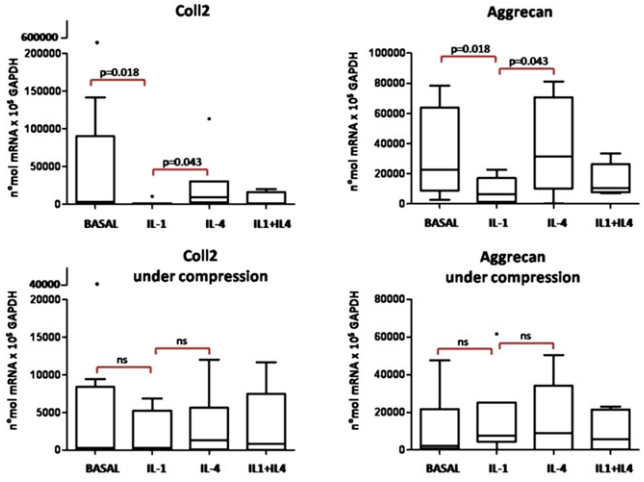


Figure. Relative mRNA expression of collagen2 (left) and aggrecan (right)
Boxes indicates 25% and 75% percentile, Whiskers indicates Min to Max values, bar indicates Median; dots indicates outliers. p value significance: p<0.05

480
MECHANICAL SIGNALS MODULATE THE C-TYPE NATRIURETIC PEPTIDE RECEPTORS VIA CGMP AND PKG DEPENDENT PATHWAYS

N. Peake [1], M. Ramachandran [2], P. Achan [2], D.M. Salter [3], D.L. Bader [1,4], T.T. Chowdhury [1]. [1] *Queen Mary, Univ. of London, London, United Kingdom;* [2] *St Bartholomew's and the Royal London Hosp., London, United Kingdom;* [3] *Edinburgh Univ., Edinburgh, United Kingdom;* [4] *Southampton Univ., Southampton, United Kingdom*

Purpose: Therapeutic agents like C-type natriuretic peptide (CNP) could be administered in conjunction with controlled exercise therapy to slow down osteoarthritis (OA) disease progression and repair damaged cartilage. For example, previous studies support a role for CNP signaling and mechanical stimuli in maintaining normal anabolic process in IL-1β treated chondrocyte / agarose constructs. The present study examined the effect of CNP and mechanical signals in modulating the natriuretic peptide receptors (NPR-B and NPR-C) and determined whether factors such as 3, 5'-cyclic guanosine monophosphate (cGMP) and cGMP-dependent protein kinases (PKG) were involved in the mechanotransduction process.

Methods: Human chondrocytes were isolated from patients undergoing total knee arthroplasty, seeded in 3 % agarose type VII (4 million cells/ml) and equilibrated in culture for 24 hrs. Constructs were subjected to dynamic compression (15 %, 1 Hz) with 0 or 100 nM CNP and / or 10 ng/ml IL-1β and / or 30 nM (D) DT-2 (inhibits PKGI) for 6 and 48 hours. In addition, PFA-fixed paraffin-embedded sections of human cartilage representing normal (grade 0-1) and OA (grade IV) were analysed for NPR-B and NPR-C by immunofluoresence and confocal microscopy. At the end of the mechanical loading experiment, cell lysates were quantified for cGMP levels by ELISA. The natriuretic peptide receptors were analysed by immunofluorescence and western blotting with monoclonal antibodies for NPR-B and NPR-C. GAG synthesis was analysed by the DMMB assay. For gene expression analysis, RNA was reverse transcribed using oligo(dT) primers and the eAMV-RT. Real-time qPCR assays coupled with LNA probes were performed with cDNA, Jumpstart® qRT-PCR Master Mix. Relative quantification of prkg1, prkg2, NPR-B and NPR-C were accomplished by normalizing each target to the reference gene, GAPDH and to the calibrator sample, by a comparative cycle threshold approach with PCR efficiencies incorporated into the analysis and data log transformed. Relevant parametric (ANOVA/post-hoc Bonferroni) tests were used to examine differences between treatment groups.

Results: The natriuretic peptide receptors were detected in sections of human cartilage by immunofluoresence microscopy. Increased staining for NPR-C was found in OA tissue and was detected further in older cartilage as shown by western blotting. Stimulation of chondrocyte / agarose constructs with CNP and dynamic compression increased gene expression of NPR-B and NPR-C in a time-dependent manner. In contrast, the presence of IL-1β inhibited gene expression of NPR-B and prkg2 resulting in enhanced gene expression of NPR-C and prkg1 and production of cGMP levels leading to reduced matrix synthesis. These effects were partially reversed by dynamic compression and / or the presence of the PKG1 inhibitor. In addition, stimulation with CNP and dynamic compression restored expression of NPR-B and prkg2 to basal levels in chondrocyte / agarose constructs cultured with IL-1β. This effect results in the production of pM levels of cGMP and restoration of matrix synthesis in chondrocyte / agarose constructs.

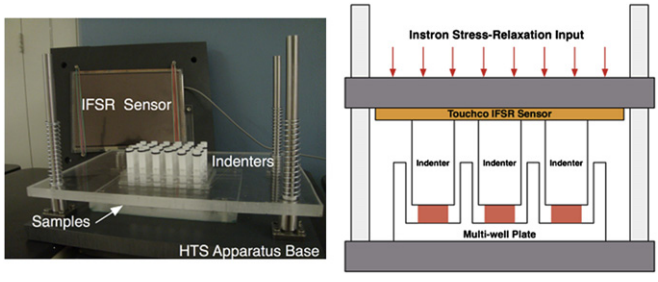
Conclusions: In summary, the present study demonstrates expression of the natriuretic peptide receptors in human cartilage which changes in OA tissue. Stimulation by mechanical signals and CNP antagonized components of the cGMP / PKG1 pathway which mediates catabolic events in chondrocytes. The catabolic effects induced by IL-1β were additionally inhibited by mechanical signals and CNP demonstrating cross-talk between the signal transduction pathways. The findings from the research will provide the potential for developing a novel agent to slow down the pathophysiological mechanisms and treat OA in the young and old.

481
A NOVEL HIGH-THROUGHPUT SYSTEM FOR THE MECHANICAL ANALYSIS AND IMPACT LOADING OF MULTIPLE ENGINEERED CARTILAGE CONSTRUCTS

B.D. Cosgrove, B. Mohanraj, C. Hou, G.R. Dodge, R.L. Mauck. *Univ. of Pennsylvania, Philadelphia, PA, USA*

Purpose: While the field of cartilage tissue engineering has made marked progress over the last decade, one major limitation is the lengthy time required to mechanically evaluate engineered constructs. Uniform mechanical impaction and testing of these materials has also not been possible on a large scale. Here, we develop a system for high throughput mechanical screening (HTMS) of engineered cartilage analogs. We set out to design a device to not only evaluate mechanical properties in a large number of samples, but also to deliver a controlled mechanical perturbation (normal and supra-physiologic) of cartilage-like constructs.

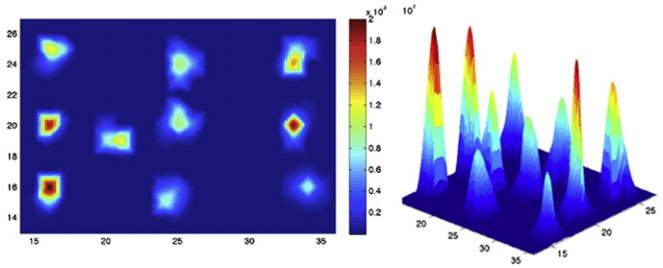
Methods: Device Components and Design: A custom HTMS device was designed using an IFSR resistive multi-touch sensor [Touchco Inc., NYC, NY] mounted in an apparatus that provides linear movement in the vertical axis (Fig 1).



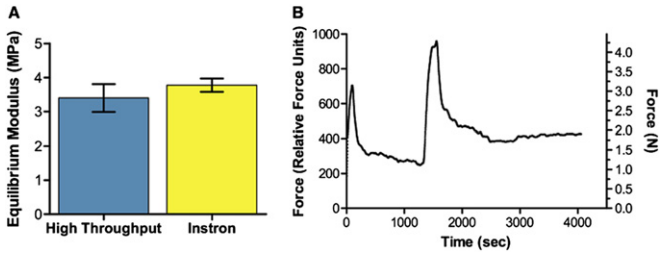
The IFSR sensor measures reaction forces generated from sample compression via 24 cylindrical PTFE indenter platens (~256 mm2 sensor area/well, 24 well plate), and total force across each well was tabulated. A traditional mechanical testing system (Instron 5543) provided overall

compressive displacement to the HT adapter (Fig 1). Protocol Development: Samples were evaluated by establishing a pre-load to ensure that all indenters were in contact with the IFSR sensor. Subsequently, a nominal 10% compressive strain was applied. With loading, the sensor sampled data at a frequency of 1Hz. Equilibrium force readings were base-lined against the initial force readings; this protocol mitigates the influence of slight differences in sample or indenter heights. Prior to testing, the IFSR sensor was calibrated to ensure accurate conversion of the relative force units outputted to engineering units (Newtons). System Validation and Sample Testing: Multiple polydimethylsiloxane (PDMS) cylinders were tested simultaneously in both the HTMS device and an Instron (with the same testing parameters). To illustrate the capacity of the device to capture time-dependent properties, bovine cartilage cylinders were evaluated by sequential stress-relaxation (5% strain, 20min hold, 10% strain, 40 min hold).

Results: The HTMS testing device accurately captured both equilibrium and dynamic reaction forces from compression of both synthetic and natural materials (Fig 2).



The modulus of PDMS samples tested individually (~3.8 MPa, n=8, Fig 3A) was slightly higher than that found for samples tested simultaneously using the HTMS system (~3.4 MPa, n=8, within ~10% of the individual measures).



As the HTMS will be used for primary screening, slightly lower thresholds for accuracy are acceptable, as secondary screens follow on from 'hits' identified in primary screens. When the device was used to mechanically compress articular cartilage, the IFSR sensor captured the time-dependent stress-relaxation response (Fig 3B).

Discussion: We have developed a HTMS platform for analysis of native and engineered tissues which also can apply a uniform compression force on multiple samples. In this prototype device, transient and equilibrium reaction forces were acquired simultaneously from up to 24 samples and allows for parallel and reliable mechanical evaluation, and in a cost effective manner (~$700). Our current HTMS device evaluates 24 samples at the same time, and is being scaled to accommodate 96- and 384-well plate designs. This validated testing platform will accelerate evaluation of mechanical properties and molecular responses to compressive injury in cartilage tissue engineering, and may help to identify disease-modifying agents in post-traumatic OA.

482

DIFFERENCES IN THE OSTEOARTHRITIC SYNOVIAL FLUID COMPOSITION AND RHEOLOGY BETWEEN PATIENTS WITH OR WITHOUT FLARE-UP. A PILOT STUDY

T. Conrozier [1], J.-C. Balblanc [1], M. Piperno [2], M. Rinaudo [3]. [1] *CENTRE Hosp.IER DE BELFORT, Belfort, France;* [2] *CENTRE Hosp.IER LYON SUD, Pierre Bénite, France;* [3] *Ctr. de Recherches sur les Macromolécules Végétales (CERMAV-CNRS), Grenoble, France*

Purpose: To study the influence of the inflammatory status (flare or not) on hyaluronic acid (HA) and protein composition and on the intrinsic viscosity of the synoviol fluid (SF) from patients with knee osteoarthritis (KOA)

Patients and Methods: Patients with KOA were classified as having flare (F+) when they fulfilled the 4 following clinical criteria: 1) sudden aggravation of knee pain, 2) whose beginning was identifiable, 3) causing nocturnal awakenings 4) with clinical evidence of knee effusion. Patients were classified F- (no flare) if they do not fulfill any of the 3 first criteria. 44 SF were obtained by arthrocentesis and assayed using Steric Exclusion Chromatography, which allows HA to be separated from the proteins and to determine both molecular weight (Mw) and and concentration (C) of both HA and proteins. SF rheology was determined using a rheometer at 25° C using a cone and plate geometry. Steady-state viscosity was determined in Pa.s, as a function of the shear rate at 1s-1. Correlations between Steady-state viscosity(Pa.s) and HA and Pr (Mw, C and Mw x C) were calculated.

Results: Among the 44 assayed SF, 25 were classified F- and 19 F+. There were statistically significant differences between F- and F+ for most of the studied variables: HA concentration and Mw (p=0.01 and 0.001 respectively), protein concentration and Mw (p 0.02 and 0.001 respectively), product Mw x C of the proteins (p<0.0001) and viscosity(p=0.0005). The product [(Mw xC) HA x (MwxC) proteins] was highly discriminating between F+ and F- (p<0.0001). The steady state viscosity was highly related to HA concentration (p= 0.0002) and HA Mw (p=0.01) and was negatively correlated with (Mw x C) proteins (p=0.0005), protein concentration (p=0.0007) and protein Mw (p=0.03).

Conclusion This pilot study shows significant differences of SF composition in patients having a flare-up compared to that of patients who do not have flare. These differences relate to both protein and HA composition and suggest that SF analysis makes possible to distinguish patients with and without flare.

483

CARTILAGE DAMAGE AFTER ACL RUPTURE; "BARCODE-LIKE LESION" AT THE MEDIAL FEMORAL CONDYLE

M. Kobayashi [1], R. Arai [1], H. Harada [1], H. Tsukiyama [1], Y. Mizuno [2]. [1] *Kyoto Univ., Kyoto, Japan;* [2] *Kyoto Katsura Hosp., Kyoto, Japan*

Purpose: This is the first report to identify horizontal articular cartilage fissures at the medial femoral condyle found arthroscopically in ACL-deficient knee, which can be called "Barcode-like lesion (BCL)". The purpose of this study is to describe BCL in details and to find out which factors correlate to its presence.

Methods: 26 cases of primary ACL reconstruction was performed between March and September, 2010. Of these, six were excluded because of lack of precise data, and twenty cases (male 17 knees and female 3 knees) with average age of 22.7±4.5 years old were enrolled in this study. Cases with meniscus tear and Grade-1 MCL injury were included. Cases with PCL and/or postero-lateral corner instability and with Grade-2 or -3 MCL injury were excluded from the study. Medical records were retrospectively reviewed regarding duration between initial trauma and the operation, pre-operative instability (side to side differences), and intraoperative findings (presence of BCL and its number, cartilage damage of tibial side, and presence of meniscal tear).

Results:BCL was found at weight-bearing portion of medial femoral condyle in 11 cases (9 males,2 females)out of 20. The number of BCL includes one in one case, two in seven cases and three in two cases. ICRS grade-IV was found in one case. Duration between initial trauma and the surgery in BCL (+) group was significantly longer than that in BCL (-) group (BCL (+), 18.9±13.3 months; BCL (-), 2.3±0.5 months;P=0.0095). All knees in BCL (-) group had intact medial meniscus, but five out of 11 BCL (+) knees had bucket-handle tear including posterior body of medial meniscus. Cartilage damage at tibial side was none or minimal. There was no difference in pre-operative instability evaluated by Telos-SE between two groups. Lysholm score of each group at one year follow-up did not significantly differ from each other. Second look arthroscopy was done in